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Review

Ethylene Oxide Gas Sterilization of Medical Devices

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Ethylene oxide gas is an agent in the sterilization of medical devices due to its effectiveness and compatibility with most materials. The advantages and disadvantages, as well as its recommended uses, are explored in this review article. The variables and their relevance on process optimization are described, the types of processing cycles are detailed and emphasis is given to the design and validation of the sterilization process.

Key words : Medical devices / Ethylene oxide gas sterilization / Process design / Process validation.

1. INTRODUCTION

Ethylene oxide gas (EOG) sterilization procedure was regarded as the last candidate of sterilization procedure due to toxic gas residue. The most recommended sterilization procedure is autoclaving, if the medical device is not sensitive to the heating. Because moist heat sterilization procedures are simple and no toxic residues. Other methods such as EOG or gamma-ray irradiation sterilization has a problem of toxic gas residue or degradation of medical devices, respectively (Shintani and Nakamura, 1991a; Shintani and Nakamura, 1991b; Shintani, 1991c; Shintani, 1992; Shintani, 1995a: Shintni 1995b; Shintani, 1996a; Shintani, 2001a; Shintani, 2004a: Shintani, 2012; Shintani, 2014a). This means EOG sterilization was not positively recommended. EOG was recognized as an anti-bacterial agent around 1929. It was initially used for sterilization of spices, thereafter it started being used as a low-temperature sterilizing agent for healthcare products (Rogers, 2005).

Nowadays, EOG is still a dominant sterilization agent used in the medical device (MD) industry due to its effectiveness and compatibility with most materials even though it has a toxicity problem. It is widely used, because it avoids heat and radiolytic stress often associated to sterilization with steam or gamma-ray irradiation. This last point is especially important due to the diversity of medical products, designs, type of materials and packaging configurations. This technique also has disadvantages, related to EOG toxicity, that require special care for the protection of workers and patients, which has led several countries to limit its use, especially in healthcare centers. This topic will be further explored.

This paper provides a framework for understanding the basic principles of EOG sterilization. The advantages and the disadvantages of this sterilization methodology and its recommended uses are described. The EOG sterilization of alkylating mechanism is explained and the variables that influence process lethality are discussed, as well as their relevance to process optimization. The EOG processing cycles are detailed and emphasis is given to the design and validation of the sterilization process, including the microbiological assessment, which is the most challenging in the validation context.

2. ADVANTAGES OF EOG STERILIZATION

EOG is an ideal gaseous sterilant because of its characteristically high diffusivity through solid matrixes (Ernest, 1973; Rogers, 2005). The main advantages of this sterilization methodology are its effectiveness and compatibility with most materials, as well as its flexibility, which results from the dependency on several factors, such as concentration, temperature, humidity, time and their combinations.

In comparison with other methods, the differential advantage of EOG is that it can sterilize heat-, moisture- or radiation-sensitive medical items without deleterious effects on the materials. For many MDs, and in particular

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thermolabile plastic, elastomer polymeric materials and most electronic devices and biomaterials. EOG is the sterilant with less degradable sterilization method (Ernest, 1973; Handlos, 1980; Shintani, 1995a; Rogers, 2005). Considering the exponential market growth of custom procedure packs that combine a diversity of products and range of polymers for use in specific medical and surgical procedures, the probability of incompatibility between material/sterilization process increases, which may be the reason for the use of EOG.

EOG sterilization is applied to the medical devices (MDs) industry, with other significant applications in pharmaceuticals and cosmetics, particularly for some chemical compounds and/or packaging materials before aseptic processing. The use of EOG as a terminal sterilization process for pharmaceuticals can be limited: (i) the EOG process might alkylate the molecules, (ii) the relatively long process times at 40℃ to 60℃ for more than 3h-24h might cause some thermal degradation (Shintani, 1995a) and (iii) components of the formulation that have low boiling points (10.7℃) might evaporate due to vacuum pulses. The penetration of EOG in liquids or powders depends upon the amount in containers: if the material is spread thin, the gas will penetrate, but this will not occur if a bulk volume is considered and this procedure is not practical. This explains why EOG sterilization is not commonly recommended for liquid or powder products due to limitation of penetration depth. This is inferior points together with toxicity residue of EOG sterilization.

The effectiveness and reliability of EOG sterilization is undeniable. The powerful microbicidal, virucidal and fungicidal activity of EOG has been demonstrated in several studies and summarized (Parisi and Young, 1991; Ries et al., 1996; Alfa et al., 1996, 1997, 1998a, 1998b; Rutala et al., 1998). The microbicidal activity of EOG is the result of alkylation of side chains of enzymes (Figure 1), deoxyribonucleic acid (DNA, Figure 2) and ribonucleic acid (RNA, Figure 2) (e.g., OH, NH_2, SH and NH). The alkylation presented in Figures 1 and 2 interferes with the normal cellular metabolism and reproductive processes, which renders a non-viability of affected microbes (Poothuill et al., 1975; Swenberg et al., 2000). EOG properties are understood, and knowledgeable users can quickly develop and validate effective sterilization processes (http://en.wikipedia.org/wiki/Ethylene_oxide).

3. DISADVANTAGES OF EOG STERILIZATION

The disadvantages associated with EOG sterilization are the lengthy cycle, the cost, and its potential hazards and toxicity to patients, staff and environment, as well

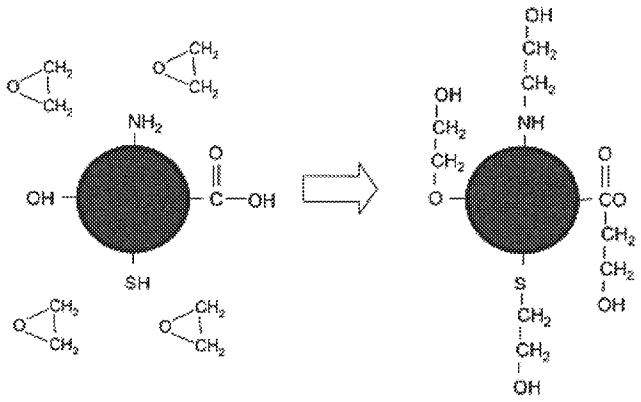

FIG. 1. Alkylation reaction with amino acid in protein and enzymes

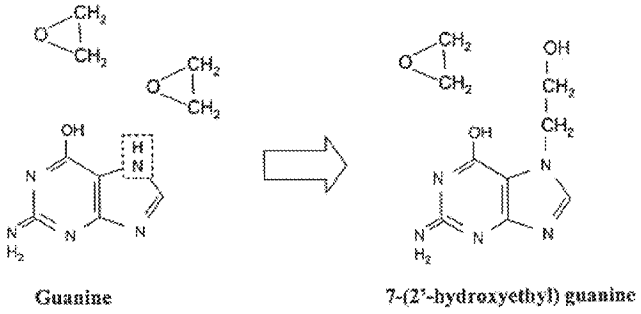

FIG. 2. Alkylation reaction with DNA or RNA

as the risks of handling a flammable and explosive gas. Due to its complexity, hazardous and toxic potential it requires a properly designed area (promoting an efficient work), sophisticated technology and equipment, feasible and ongoing engineering controls, safe work practices and trained staff. Detectors are required to protect staff workers, especially since this gas is colorless and odorless until a level of 430 ppm. Moreover, careful aeration of EOG sterilized MDs is required since absorbed EOG can leave toxic residues on them. The residue is not limited to EOG. Byproducts must also be seriously concerned as mentioned later. EOG disadvantages have been overcome by equipment and facilities investments, which have improved the process efficiency while guaranteeing workers' security and environmental protection. The processing equipment consists of tightly closed, mostly automated and controlled systems. Currently, the EOG sterilizers combine sterilization and aeration in the same chamber or in a continuous chamber in automatic communication with the sterilizer, achieving a nonstop process that minimizes the potential occupational exposure to EOG. The AAMI TIR 15 standard (2009, http://marketplace.aami.org/eseries/scriptcontent/docs/Preview%20Files/TIR150909_preview.pdf) provides guidelines for design and

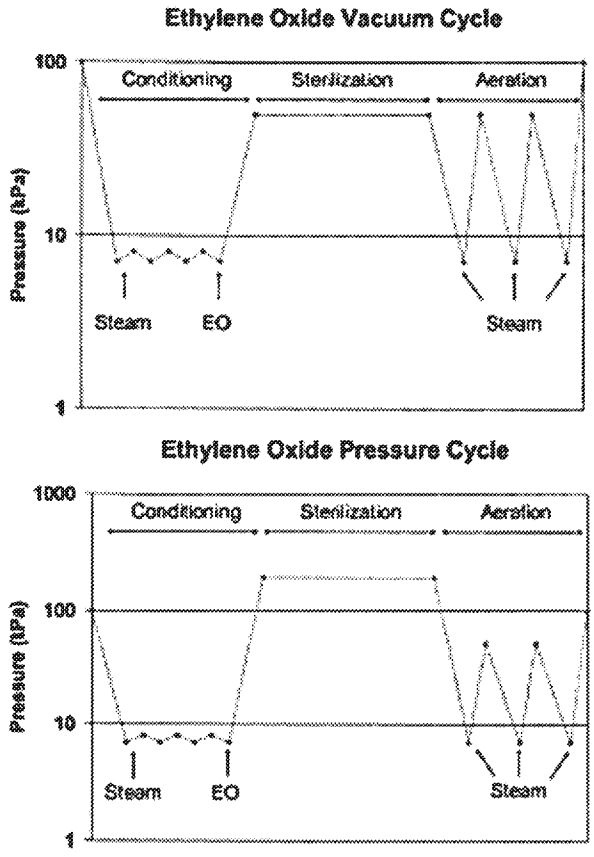

FIG. 3. Typical EOG processes

selection of an appropriate sterilization equipment and facility for attaining a safe, reliable and effective process. The EOG sterilization process is complex. This is due to the EOG inherent toxicity and flammable properties, as well as the large number of variable parameters involved. It requires knowledge and careful monitoring, an efficient sterilization process can be achieved by skilled technicians.

The capacity of EOG sterilizers varies from table-top size to very large floor-loading chambers (Figures 3 and 4), but, due to the inherent risks associated with EOG, this technology is becoming more and more industrial and its use in health care units is decreasing due to the toxic residue and the less skilled technicians.

Its complexity, numerous speculative risks and misconceptions led to unfair criticism and disapproval of the EOG process. Despite many predictions about its demise as a sterilization procedure, it is still a mode of sterilization and it continues to be used for MDs because UD FDA declares that EOG sterilization procedure is the last candidate to select. However, EOG can be used safely with minimal hazard and its benefits continue to outweigh its inherent risks (ANSI/AAMI ST 41, 2008, http://marketplace.aami.org/eseries/scriptcontent/docs/Preview%20Files%5CST410810_preview.pdf).

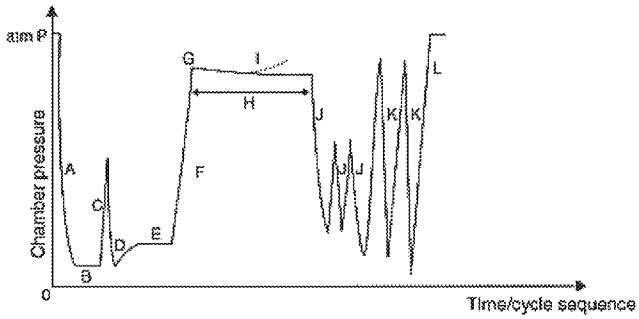

FIG. 4. Typical 100% EOG process cycle

3.1 Time and cost

The extended process time of EOG sterilization is mainly due to quarantine period of the biological indicators (BIs) required for clearance and approval (ISO 11138-2, 2006), conventional release, and aeration time for EOG residues removal (ISO 11135-1, http://marketplace.aami.org/eseries/scriptcontent/docs/Preview%20Files/11135010707preview.pdf). Recent technological advances have greatly reduced the cycle time of EOG sterilization, due to the development of aeration processes optimization. Proper EOG handling requires sophisticated equipment, automatic controls that preclude human error and careful monitoring, which results in high operational handling cost. This topic will be further discussed.

3.2 Risk to patients and workers

The large variability of the rate and extent of EO adsorption and desorption by the different polymers used in the MD industry requires careful verification that EOG residues and by-products in MDs are below hazardous levels before their use on the patients. The IS0 10993-7 (2008) specifies the allowable limits for residual EOG and for its by-product, ethylene chlorohydrin (ECH) and ethylene glycol (EG), which is formed due to the EOG reaction with HCl aqueous solution or H_2SO_4, aqueous solution, respectively. EG was not produced by water alone. If water alone can change EOG to EG, water extraction described later cannot be applicable as an extraction solvent of EOG. Please keep this in mind.

These chemicals are particularly relevant, since the exposure to devices that have been improperly aerated can cause irritation and, eventually, burns to skins and mucosa and so on (ANSI/AAMI ST 41, 2008). Concerning EG, no exposure limits are defied (previously it was defined as 250 ppm in ISO 10993-7) because studies have shown that when EOG residues are controlled, it is

likely that residues of EG would be present due to hydration to EOG in human body, but the toxicity of EG is more than 10 times that of EOG or ECH, therefore may be omitted in ISO 10993-7 (ISO 10993-7, ANSI/AAMI ST 41, 2008, http://www.biochem-bcm.com/ISO%2010993-7%20Sampling.pdf and http://marketplace.aami.org/eseries/scriptcontent/docs/Preview%20Files%5CST410810_preview.pdf).

The limits for EOG residues were established using health-based risk assessment studies (25 ppm, aiming at a minimal risk to patients during standard use of the product), taking into account the contact time with the device (limited exposure -daily; prolonged exposure -monthly; permanent exposure; although certain exceptions occur for particular device). The ISO 10993-7 (2008) also outlines suitable methods for the extraction of residues from products (using exhaustive extraction and simulated-use procedures), details the subsequent analysis via gas-liquid chromatography (GLC) and provides the procedures for determining compliance and subsequent sterilized MD release to the market. The extraction solvent is water and olive oil, both are not resemble characteristics to human blood (http://www.fda.gov/downloads/medicaldevices/deviceregulationandguidance/guidancedocuments/ucm348890.pdf). The author recommends to use automated solid phase extraction with the extraction solvent of blood serum (Shintani, 1991d; Shintani, et al., 1993: Shintani, 1996b; Shintani, 2001b; Shintani, 2014b; Shintani 2014c). The objective of simulated-use procedures is to quantify the bioavailable EOG residues, which is the amount of EOG that may be assimilated by the body; however in ISO 10993-7, water extraction is recommended to carry out under conditions that represent the intended use of product (at room temperature, 22℃ or body temperature, 37℃). The amount of water extraction was significantly smaller than that of blood extraction, therefore water extraction was under-estimation compared with blood extraction. Water extraction of EOG does not produce EG if not protonated by acid to EOG like $(CH_2)_2OH^+$, but blood extraction produces EG because blood contains several organic or inorganic acids, which both significantly differ. With exhaustive extraction (thermal extraction followed by headspace analysis and solvent extraction procedures, with either headspace gas analysis or chromatography of the solvent extract), the intention is to recover the entire residual content of a device. Simulated-use methods are commonly used for devices with limited potential patient exposure, while exhaustive methods are appropriate for prolonged or permanent exposure devices.

It is important to carefully study the method for residuals quantification. There are no general rules and each specific material has its own characteristics (AAMI TIR 19, 1998, 1999; IS0 10993-7,2008). Tests can be conducted at the final desired aeration time-point, or an EOG dissipation curve can be established by periodic sampling and analysis of the product. Release is based on the time after sterilization when the regression line intercepts the maximum allowable residue (Shintani et al., 1981a and 1981b). These data can be used to establish quarantine times prior to product release, or to provide additional information about the influence of manufacturing, packaging or sterilization processes on product EOG 1evels. The adsorption and degassing of EOG from sterilized products is influenced by several factors (Shintani and Oba 1981b), and the conditions under which degassing occurs have a high influence on EOG residues diffusivity. This issue will be further discussed in a later section (IS0 10993-7, 2008).

Despite well-known EOG toxicity (http://www.atsdr.cdc.gov/mmg/mmg.asp?id=730&tid=133), there are large uncertainties associated with the current quantitative risk assessment studies that establish the undesirable effects of EOG residues on patients' and workers' health. However, the most important is that EOG potential risks were always estimated conservatively, which means that its effects may be overestimated. The residues of EOG should be kept as low as feasible, and cannot exceed the limits defined by ISO 10993-7 (2008); EOG potential risks become trivially small if doses are significantly low (Mendes et al., 2007; 2008).

3.3 Workplace considerations

When EOG sterilization equipment is elected, provisions should be made for compliance with Occupational Safety Health Administration (OSHA) safety standards and state regulations. Workplace exposure to EOG is regulated by OSHA through standard 29 CFR 1910.1047 (ANSI/AAMI ST 41, 2008; AAMI TIR 15, 2009).

3.3.1 Health Risks

Acute overexposure to EOG may result in irritation (e.g., to skin or mucous, eyes, gastrointestinal or respiratory tracts) and central nervous system depression. Chronic (long term) exposure to EOG has been linked to an increased risk of cancer and reproductive effects, neurotoxicity, fetotoxicity and spontaneous abortion (http://www.atsdr.cdc.gov/toxprofiles/tp137.pdf). In various *in vitro* and animal studies, EOG has been demonstrated to be carcinogenic; findings in humans and experimental animals exposed to EOG airborne concentrations also indicate damage of the genetic material (DNA, RNA), due to its alkylating properties (Figures 1 and 2). Currently, limited studies on chronic effects in humans, resulting from exposure to EOG, suggest a causal association with leukemia. As being classified by the Environmental Protection Agency (EPA) to Group

B1 (probable human carcinogen), recent epidemiological studies of controlled occupational exposure to EOG did demonstrate potential cancer risk in workers (29 CFR Part 1910.1047, n.d.; ANSI/AAMI ST 41, 2008; Valdez-Flores et al., 2010), therefore EOG risk is seriously problematic to be considered.

3.3.2 Occupational exposure limits

Workers' exposure to EOG should be kept as low as feasible. OSHA has established a permissible exposure limit (PEL) of 1 ppm airborne EOG in the workplace, and an action level of 0.5 ppm, expressed as a time-weighted average (TWA), for an 8 h work shift in a 40 h work week. Exceptionally, exposures above I ppm are allowed if they are compensated by equal or longer exposures below the limit, during the same 8 h work day. The short-term exposure limit (STEL) is 5 ppm, expressed as a 15 min TWA, and OSHA has also established a PEL of 5 ppm for ethylene chlorohydrin (ECH) in the workplace (ANSI/AAMI ST 41, 2008). Workers who are or will be exposed at or above the action leve1 (0.5 ppm) for 30 or more days per year should be submitted to medical examination and clinical analysis control, at least annually (ANSI/AAMI ST 41, 2008).

3.3.3 Environmental and employee monitoring

In order to ensure a safe and healthy work environment and to establish compliance with regulated limits and voluntary guidelines on occupational exposure to EOG, airborne EOG concentrations must be monitored in the workplace. Two general types of monitoring are performed in EOG sterilization facilities: personnel monitoring (devices used by operators) and area monitoring.

Personnel monitoring aims at determining airborne contaminants in the employee breathing zone (EBZ), which is assumed to be the amount actually inhaled. The two most popular methods that have been used for EOG exposure determination are charcoal tubes and passive dosimeters. Tedlar^{R} gas-sampling bags (http://www.sigmaaldrich.com/analytical-chromatography/analytical-products.html?TablePage=16369234), impingers and detector tubes are examples of other personnel monitoring systems. In addition, there are several commercially available real-time continuous monitoring analyzers, portable and directly readable.

Area monitoring is performed for determination of environmental EOG concentration in a particular workplace area. The following types of area monitoring devices are currently available: metal oxide semiconductors, electrochemical sensors, GLC, FT-IR (http://pubs.acs.org/doi/abs/10.1021/ac60116a002), photoionization detectors and gas detector tubes. The continuous monitoring of the work place environment can also be interfaced with controls to increase ventilation when the OSHA action level, PEL or STEL is exceeded.

Each method has its own specific limitations and this topic is explored in ANSI/AAMI ST 41 (2008).

3.3.4 Personal protective clothing and equipment

If eye or skin contact with EOG or EOG mixtures might occur, such as during sterilizer maintenance, EOG cylinder changing, or by EOG leak or spill, appropriate personal protective equipment (PPE) must be used (29 CFR Part 1910. 132; 29 CFR Part 1910.133).

When excessive EOG exposure could occur, personnel should use an adequate respirator, certified by the National Institute for Occupational Safety and Health (NIOSH). The handling of liquid EOG requires impermeable clothing (coveralls or similar full-body work clothing, g1oves, head covering, face shields or splash-proof safety goggles) and impermeable shoes. Rubber and leather must be avoided, since liquid EOG readily penetrates these materials (ANSI/AAMI ST 41, 2008).

3.4 Environmental Impact

The regulations by the EPA must be followed in order to control the potential environmental risks. In addition, the risks associated with handling a flammable and explosive gas also need to be considered.

3.4.1 Emission control systems

Several countries have introduced regulations to limit the amount of EOG released in the atmosphere (i.e., Guidelines for the Reduction of Ethylene Oxide Releases from Sterilization Applications, Environment Canada). The most important systems for reducing EOG emissions are catalytic converters and acid water scrubbers. The first system is the most efficient and operates at relatively low temperatures (121-288℃) to namelessly convert EOG to carbon dioxide (CO_2) and water vapor. The second one basically consists of a bath where effluent EOG reacts with acid water, converting it into EG. Others for example EOG is catalyzed to ethanol (http://www.sciencedirect.com/science/article/pii/S0021951710002162).

In addition, absorption systems (e.g., filtering media) can be used to absorb EOG in low concentrations and some systems also operate with recovery (or reclamation), which means that the gas is reprocessed for reuse rather than discharged into the atmosphere. Besides the reduced costs of this procedure, it is not common due to its inherent complexity and associated risks (ANSI/AAMI ST 41, 2008).

3.4.2 Recommendations for working with a flammable and explosive gas

EOG is flammable and can be highly explosive when pure. Its range of flammability, as a mixture in air,

extends from 3.6% to 100% by gaseous volume. When 100% EOG or flammable blends of EOG are used, electrical accessories should comply with Class I, Division 2, Group B electrical requirements, as stated by the National Fire Protection Association (NFPA) in NFPA 70 (2008) or equivalent; the sterilizer interior should comply with Class I, Division 1, Group B electrical requirements stated within NFPA 70 (2008) or equivalent; the equipment and piping should be grounded in accordance with NFPA 70 (2008) or equivalent. In facilities constructed after 1995 and where NFPA standards are under a jurisdiction, the storage, handling and use of EOG should comply with NFPA 560 (2007).

It is recommended that the chamber environment should remain within the non-flammable zone; therefore, the flammability calculations should be considered when designing sterilization cycles (AAMI TIR 15, 2009). The mixture of EOG and chlorofluorocarbon-12 (CFC-12), referred to as 12/88 EOG (mixture of 12% EOG and 88% CFC-12) and most commonly used in the late twentieth century, was banned in December 1995 under provisions of the Clean Air Act. The scientific evidence that linked the gas mixture to the destruction of the earth's ozone layer was the basis of the decision (ANSI/AAMIST 41, 2008).

Nowadays, EOG cycles with nitrogen are common and sterilant mixtures of EOG with hydrochlorofluorocarbons (HCFCs) or of EOG with CO_2 can also be used to reduce the potential flammability of EOG. HCFCs also cause some depletion of the earth's ozone layer, although to a lesser extent than CFC-12 due to resemble chemical structure, and the international agreements call for it to be phased out completely in 2015 (ANSI/AAMI ST 41, 2008; AAMI TIR 15, 2009).

3.4.3 EOG processing cycle

EOG may be used pure or diluted with HCFCs or CO_2, and these latter solutions are neither as effective nor as cost efficient as 100% EOG. 100% EOG use associates explosive problem, so that ceiling is fragile towards explosion. Typically and mostly in US, not in Japan, large-scale industrial units use pure EOG (Figure 4), while the blends are in general used in smaller laboratories and in healthcare facilities. In Japan in general pure EOG should not be used due to explosion, but blends with 80% CO_2.

The typical EOG processing cycles are (29 CFR Part 1910.1047; Ernest, 1973; Rogers, 2005; AAMI TIR 17, 2008; AAMI TIR 15, 2009):

1) 100% EOG cycles with/without nitrogen. This is the typical industrial cycle in several countries except Japan. Its advantages are related to its lower cost (than the non-flammable blends), its adequacy for sensitive materials (due to lower damage) and to the reduction of potential hazards due to environmental EOG exposure (potential gas leakage is minimized). In addition, despite requiring intrinsically explosion-proof equipment and instrumentation, this solution does not require a pressure vessel, since chamber pressures are below atmospheric.
2) Standard EOG/HCFC cycles. These non-flammable gas mixtures provide safe working conditions and this solution is useful in non-explosive facilities. Their use is being restricted due to the ozone depletive properties of HCFCs. The common blends are: (i) 8.6%w EOG/91.4%w HCFC-124, (ii) 10%w EOG/90%w undisclosed HCFCs and (iii) 10%w EOG/27%w HCFC-22/63%w HCFC-124.
3) EOG/CO_2 (high-pressure) cycles. These non-flammable gas blends are less expensive than EOG/HCFC blends. The disadvantage of this mixture is the high-pressure process that is required to achieve an effective sterilization concentration, and the inherent reduction of the EOG sterilization efficacy. The common blends arc: (i) 8.5% EOG/91.5% CO_2 (w/w), (ii) 20% EOG/80% CO_2 (w/w) and (iii) 30% EOG/70% CO_2 (w/w). Among these alternative choice, the (ii) is the most popular in Japan.

4. STERILIZATION PROCESS CHARACTERIZATION

The basic EOG sterilization cycle consists of five stages -that is, preconditioning and humidification, gas introduction, exposure, evacuation and air washes (ISO 11135-1, 2007).

4.1 Preconditioning area (outside sterilizer chamber)

The preconditioning facilities (typical in industrial processes) provide heat and humidification to the product and to bioburden through assisted air circulation, shortening the cycle time and equalizing the temperature and humidity of the loads. The time required for adequate temperature and humidity balance of the load should be evaluated in the coldest seasons or by using a refrigerated load to simulate the lowest temperature to which the product may be exposed before preconditioning (ISO 11135-1, 2007; AAMI TIR 15, 2009).

4.2 Typical industrial sterilization cycle

Vacuum cycles are the preferred choice and the use of pure EOG as sterilizing agent, together with nitrogen as inert gas, is increasing in US, not in Japan. The basic steps of a hypothetical and typical 100% EOG sterilization cycle, also called deep vacuum cycle, are explained in

Figure 4 and a short description in terms of cycle optimization is also given in the following.

(A) Air removal-vacuum

Air is withdrawn from the chamber. This step is necessary so that subsequent EOG injection will not pass through significant flammable limits or explosive conditions, and the deeper the vacuum, the higher the moisture diffusion.

(B) Leak test

The chamber tightness is checked before injecting EOG. The leak test is carried out using soap bubbling.

(C, D) Nitrogen flushes.

Nitrogen injections and evacuations can be used to reduce the oxygen concentration in the chamber. Inert atmospheres are attained, which are safe for EOG injection.

(E) Conditioning- steam injection for temperature and humidity stabilization

The purpose of humidification is to drive the moisture deep into and through the materials and heat up the sterilization load. Humidification is performed under vacuum and prior to admission of EOG because the water vapor molecules diffuse slower than the EOG molecules. Humidification can be achieved by static or dynamic environmental conditioning as follows.

1) Static humidification: steam is injected into the sterilizer until a certain pressure, or a target relative humidity level, is achieved in the sterilization chamber. During the humidity stabilization, as the load mass adsorbs the injected moisture, chamber pressure is maintained by steam injection.
2) Dynamic environmental conditioning (DEC): DEC is a more effective process designed to heat up the load using flowing steam as the heating medium. The amount of heat available is dependent on the operational pressure during the DEC phase. This process follows a steam-bleed principle, because steam is injected in a steady flow as the chamber is being evacuated, and in this way the air is removed or displaced by the action of steam injection.

Two common methods for delivering DEC are pulsed-steam injection (also known as stepped conditioning because vacuum pull is alternated with steam injection) and continuous steam injection because a steam injection is kept constant while the vacuum pump is activated.

(F) Sterilant injection

EOG and nitrogen injections are in equilibrium in order to provide the required EOG concentration and a non-flammable mixture.

(H, I) Exposure time

A forced recirculation is important for keeping homogeneous sterilization conditions during this step. If the original chamber pressure is to be maintained throughout the exposure, it can be done by using either EOG or inert gas/nitrogen make-ups or additions. When nitrogen is used to maintain the chamber pressure, the recirculation system will be sufficient to minimize the risk of the inert and EOG gases stratification, and to avoid the potential effect of reduced EOG concentration which could affect the lethality rate.

(J) Flushing-nitrogen rinsing step

The EOG is purged to remove the residual sterilant and the chamber is flooded with nitrogen to keep inert atmospheres inside the sterilizer; successive operations may be performed.

(K) Flushing

The EOG is removed from the chamber by consecutive vacuums and injections of filtered sterile air.

(L) Air admission

Last air rinsing brings the chamber back to atmospheric pressure.

4.2.1 Aeration

Aeration can be performed in the sterilizer or in a separate aeration chamber or room, under controlled conditions (Figure 5). All EOG sterilized materials

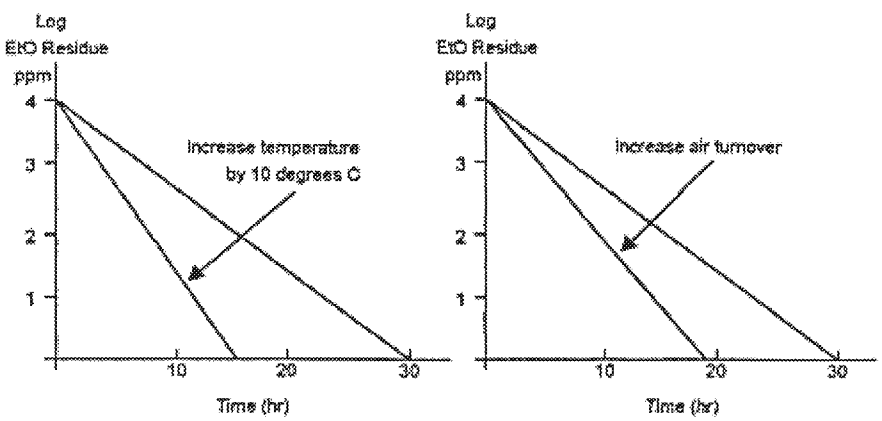

FIG. 5. Aeration vs temperature (left) or turnover (right)

should be properly aerated before handling and use. The aeration time depends on many variables including:

1. composition, density, porosity, dimensions, surface area and design configuration of the material. Metal and glass are two materials that retain very low EOG quantities. Polymers adsorb and desorb EOG at relatively high but variable rates. Polyvinyl chloride, polystyrene and rubber retain more EOG than polyethylene, polyurethane, silicone, acrylic butyl styrene and polycarbonate, which retain more EOG than nylon, paper or cotton. In general, hydrophilic material can retain more EOG than hydrophobic material because EOG is hydrophilic.
2. packaging material (wrapping material and/or sterilization container system);
3. sterilizing conditions (i.e. temperature, sterilant concentration, exposure time);
4. aeration conditions;
5. size, configuration of the load, and number of highly EOG-absorptive materials being aerated; and
6. acceptable limits of residues for the intended use of the MDs (Ernest, 1973; Handlos,1980; Scott, 1982; Aeschlimann, 1984; Muzeni, 1985; Vink and Pleijsier, 1986; Buben et al., 1999; Lucas et al., 2003; Rogers, 2005; Mendes et al., 2007: 2008; ISO 11135-1, 2007; ANSI/AAMI ST 41, 2008; ISO 10993-7, 2008; AAMI TIR 15, 2009; AAMI TIR 16, 2009).

4.3 Process variables

The EOG sterilization is a complex multi-parameter process. The effectiveness of the EOG sterilization process is influenced by many variables and each one may be varied, this affecting the other dependent parameters. An effective process design requires an understanding of the process parameters and the interrelationships between them and the products. The most significant variables are outlined below (ANSI/AAMI ST 41, 2008; AAMI TIR 17, 2008; AAMI TIR 16, 2009).

4.3.1 Pressure

Initial vacuum level interferes with the sterilization efficacy because the residual air in the load hinders moisture diffusion, and consequently affects heat and gas transfer into the product. Besides the pressure depth, the process specification also involves the establishment of the gas injection and evacuation rates due to their effect on the cycle lethality, as well as due to the potential for package and product damage.

Shallow vacuum processes (nitrogen soft cycles), in which the vacuum levels are at or around 1/2 of atmospheric pressure, are designed for sterilizing pressure-sensitive materials. Deep vacuum processes are adequate for sterilizing loads that do not contain pressure-sensitive materials.

4.3.2 EOG concentration

The EOG concentration can be measured by FT-IR, head space GLC and microwave spectroscopy, or can be calculated (Mendes et al., 2007; AAMI TIR 15, 2009). The higher the concentration, the faster the sterilization process; however, higher concentrations will lead to higher EOG residuals and consequently to increased aeration times. Since this variable interferes with the microbial inactivation kinetics and with outgassing, the process optimization also requires considerations about the material (i.e. EOG absorption and retention characteristics).

As the EOG concentration increases from 50 to 500 mg/L, there is a significant increase of the microbial death rate. At concentrations above 1,200 mg/L, the rates do not increase significantly (Figure 6). The use of concentrations between 400 and 650 mg/L is recommended for achieving microbiological lethality in most products within a reasonable and practical exposure time, and without disregarding the EOG residuals. In general 500 mg/L is used popularly in Japan.

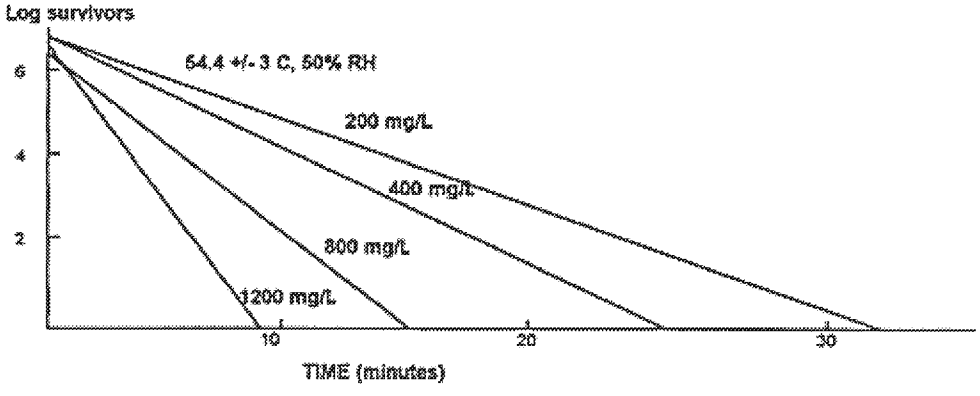

FIG. 6. EOG concentration vs sterilization efficiency

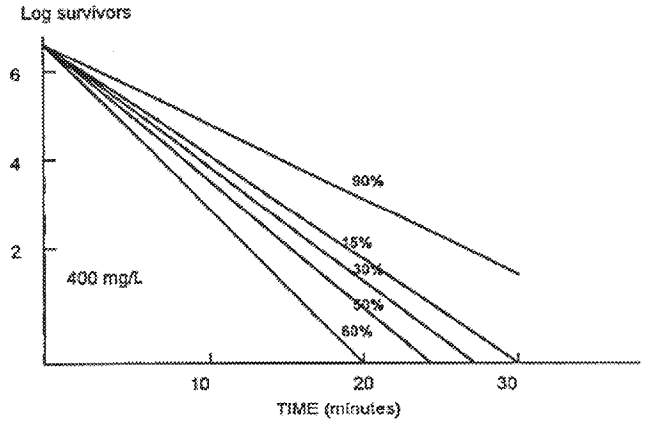

FIG. 7. Humidity vs sterility efficiency

4.3.3 Temperature

The temperature has a significant influence on microbial lethality and affects the EOG diffusion through cell walls and packaging materials. High-density loads and items composed of materials with low thermal diffusivity require longer heat-up time.

Microbial death rate depends on temperature and, consequently, if high temperatures are used, the cycle time can be reduced. However, it is important to consider the maximum temperature the product and the package can withstand. Typical operational temperature values are above 35 to 60℃. It is consensual that a O_{10} value of 2, which means that a 10℃ change would affect lethality by a factor of 2.

4.3.4 Humidity

Relative humidity may be directly measured or calculated (AAMI TIR 15, 2009). This parameter plays a critical role in EOG sterilization processes and is the most complex and difficult factor to determine of the controllable variables because it influences the gas diffusion. An inadequate humidification is the major contributory cause for most microbiological failures of EOG processes. There do not exist any humidity detector to determine humidity correctly and reproducibly under the EOG circumstances.

A level of relative humidity (RH) above 30-35% and below 85-90% in the chamber is commonly used to achieve an effective EOG sterilization (Figure 7) and particular consideration should also be taken due to product limitations. Excessive moisture should be avoided throughout the cycle because it inhibits sterilization (drops of water protect microorganisms from EOG action).

4.3.5 Exposure time

The time necessary to provide the required sterility assurance level (SAL) is primarily related to gas concentration and temperature. It should be taken into consideration that the lethality occurs not only during the exposure time, but also during the sterilant injection time (this including the nitrogen blanket injection, if used) and the sterilant removal time.

4.3.6 Aeration

Aeration after processing is important for the removal of EOG residuals (Figure 5). Temperature (usually between 37-50℃), dwell time, rate and number of air changes, air flow rates and patterns (conditioned by the loading characteristics) will affect the EOG diffusion from the product load. Different aeration technologies are known as shown in Figure 5, such as pulsed vacuums post-process and heat addition, steam addition and removal combinations of different gases and pressure set points, and newer developments, such as microwave desorption.

4.3.7 Packaging

The product packaging should be permeable to gas and humidity, should allow aeration after cycle completion and should be capable of tolerating vacuum/pressure differentials and evacuation/pressurization rates. The material itself, the layers of packaging (number of barriers) and the material density influence permeation.

4.3.8 Device

The type of materials, complexity and design of the devices influence the EOG and humidity penetration.

4.3.9 Load

The load density and the configuration influences the EOG and thermal diffusion. The load capacity is less than 80% of the sterilization chamber volume to make EOG penetration easier and even throughout the interior chamber.

4.3.10 Microbial contamination

It is important to keep the cleanliness of the device itself and of the packaging under control. An environmental monitoring program should be established to monitor the cleanliness levels (Ernest, 1973; Rogers, 2005; Mendes et al., 2007; AAMI TIR 17, 2008; ANSI/AAMI ST 41, 2008; AAMI TIR 15, 2009; AAMI TIR 16, 2009).

5. PROCESS DEFINITION

The cycle development studies are to attain a desired microbial lethality in the product, while maintaining its functionality and safety, as well as package integrity. These studies may be conducted in a small development vessel or in a large production chamber. The use

of a research sterilization vessel provides a more effective process control and easier and faster sample removal such as BIER (biological indicator evaluator resistometer) (ISO 11135-1, 2007; AAMI TIR 17, 2008; AAMI TIR 16, 2009). BI provides a unique direct measure of the process lethality. The bacterial spore, especially *Bacillus atrophaeus* ATCC 9372 is the most resistant microorganism and consequently it is the recommended BI (Mendes et al., 2007; ANSI/AAMI ST 41, 2008; ISO 11138-2, 2006, http://marketplace.aami.org/eseries/scriptcontent/docs/Preview%20Files/11138021004_preview.pdf).

5.1 Lethality modeling

The mathematical modeling of the EOG sterilization cycle allows the definition of optimal inactivation conditions, which is particularly important for industry. The accurate prediction of D values and process times, required for a target SAL, allows cycle times and/or EOG concentration reduction, as well as the comparison of effectiveness and equivalency of different sterilization processes. Furthermore, lethality modeling contributes to process efficiency and flexibility, and the parametric release is not much more supported due to too many parameters determined as mentioned above. Especially, determination of relative humidity is quite hard to attain reproducibly due to EOG polymerization.

In order to integrate mathematically the dynamic temperature and concentration conditions effects on inactivation, it developed the following model for BI spores of *Bacillus atrophaeus* ATCC 9372 reported in URL of http://www.mddionline.com/article/calculating-accumulated-lethality-and-survivorship-eto-sterilization-processes. Rodriguez et al (2001) reported the equation specified to *Bacillus atrophies* ATCC 9372.

Mosley et al., (2002) deduced an alternative model for equivalent process time prediction. Please refer URL of http://www.mddionline.com/article/calculating-equivalent-time-use-determining-lethality-eto-sterilization-processes for citation.

The mathematical models above presented in two citations are essential for designing EOG sterilization processes. Optimization and validation of the different methodologies are presented by Shintani, 2006a and Mendes et al., 2007.

5.2 Microbiological methods

The four approaches for microbial lethality assessment are: half-cycle, overkill, combined BI/bioburden and absolute bioburden methods (ISO 14161, 2009; http://www.google.co.jp/search?hl=ja&source=hp&q=iso+14161+pdf&gbv=2&oq=ISO+14161&gs_l=heirloom-hp.1.1.0j0i30l3j0i5i30l6.1886.7401.0.9940.15.12.3.0.0.0.254.1314.9j2j1.12.0.msedr...0...1ac.1.34.heirloom-hp..0.15.1343.XKazqKHgHQU). By this order, the complexity and the processing period decreases, indicating the 1st is the longest.

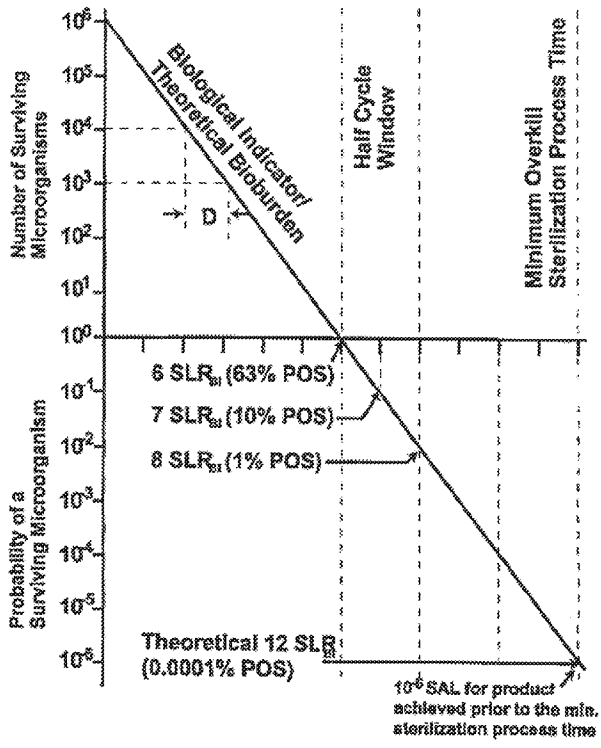

FIG. 8. Fraction negative method

The selection of the method for estimation or calculation of the cycle lethality is also part of the process, using either the fraction-negative or direct enumeration method (also known as survivor curve method in ISO 11138-1). Fraction-negative analysis involves running sterilization cycles in which some, but not all, of the BIs are inactivated (Figure 8). The proportion of viable and non-viable BIs allows D-value calculation by Spearman Karber Procedures (SKP), Limited SKP (LSKP) and Stumbo Murphy Cochran Procedure (SMCP) and Limited Stumbo Murphy Cochran Procedure (LSMCP) (ISO 14161, 2009; Shintani, 1995c). The enumeration method consists of counting the surviving organisms on each BI, using a serial dilution/plate count method up to 30-300 cfu/plate (ISO 14161, 2009). For both situations, the bioburden recovery and sterility test methods should be validated to ensure recovery of injured organisms (ISO 11135-1, 2007; ISO 11138-2, 2006; AAMI TIR17, 2008; AAMI TIR 16, 2009). Even though ISO documents on injured microorganisms are presented, but at ISO meetings the injured microorganisms have rarely discussed (Shintani, 2006b; 2013b).

5.2.1 Evaluation of product bioburden

An understanding of the viable microbial population

on MDs (bioburden, sort and number of viable microorganisms existing in/on the products) is necessary and required to support the validation process. The following methods have been used to estimate the natural bioburden on the product and its sort and number are compared with the BI (ISO 14161, 2006). In order to estimate resistance of bioburdens, it is necessary to use BIER (http://www.pharmtech.com/node/233664?rel=canonical, ISO 14161, 2006). The estimated time to inactivate the bioburden for 1 log reduction is D value of the bioburden.

The chosen product samples should be representative of the product family having the highest or the most resistant bioburden, and several different products can be tested if there is more than one high-bioburden family group (ISO 14161, 2006; ISO 11737-1, 2006).

1) Half cycle approach

Due to its relative ease of use and the robust SAL obtained, the half cycle approach is the most widely used method to validate MDs by EOG sterilization. In this approach, more than 6 spore log reduction (SLR) of a 10^6 CFU/carrier BI is attained (by achieving sterile BI samples); therefore, if exposure time is doubled, more than 12 SLR (so often 12-16 SLR process) might occur (Figure 8). (Mosley et al., 2002, 2005; Mosley and Houghtling, 2005).

Half cycle method can be applied only for EOG sterilization because not so much degradation of the products can be observed after sterilization. Other sterilization methods never utilized half-cycle methods to avoid deterioration of the sterilized MDs.

2) Overkill method

The overkill approach uses BI data to assess the microbial inactivation rate for a given process. The overkill method is applicable for 12 D because initial population was 10^6 CFU/carrier and the SAL was 10^{-6}, thus as a whole 12 log reduction. D value is calculated within the sterilization chamber and BI is set at the most difficult to sterilize place in the chamber verified by validation study. BI is commercially available BI defined by ISO 11138-2 and ISO 14161.

3) Combined BI/bioburden method

If the product bioburden is routinely tested and if the microbial population is low, then a combined BI/bioburden method can be used for cycle development. This method is based on the assumption that the bioburden is less (or equal to) resistant than the BI. Combined BI/bioburden method defines the treatment extent required to achieve the SAL of 10^{-6} and the BI is *Bacillus atrophaeus* ATCC 9372 and the initial population is the bioburden number or more than 10^3 CFU/carrier (ISO 14161, 2006), which the latter can be purchased commercially available BI defined in ISO 14161.

TABLE 1. Bioburden microorganisms identified in the Namiki Clinic dialysis room

Bacterial species	cfu
Staphylococcus haemolyticus	3
Staphylococcus hominis	2
Staphylococcus schleiferi	2
Staphylococcus epidermidis	7
Staphylococcus intermedius	1
Staphylococcus saprophyticus	2
Staphylococcus capitis	1
Staphylococcus cohnii sub sp. cohnii	1
Staphylococcus pasteuri	1
Staphylococcus vitulus	1
Streptococcus sanguis	1
Micrococcus luteus	6
Micrococcus sedentarius	6
Micrococcus species	4
Bacillus licheniformis	4
Bacillus subtilis	7
Bacillus megaterium	1
Acinetobactor lwoffii	2
Lactobacillus raffinolactis	1
Actinomyces pyogenes	3
Saccharomyces species	1
Corynebacterium genitalium	1
Gardnerella vaginalis	1
Pantoea agglomerans	1

cfu: colony forming units, Data for fungi, molds and yeast other than *Saccharomyces* are omitted.

4) Absolute bioburden method

This method is based on the assumption that the bioburden is less (or equal to) resistant than the BI. Absolute bioburden method defines the treatment extent required to achieve the SAL of 10^{-6} and the BI is the most tolerable bioburden microorganisms and the initial population is the bioburden number (ISO 14161, 2006), thus BI is self-made.

The example of bioburden is presented in TABLE 1 (Shintani, 2004b; Shintani, 2013a).

6. PROCESS OPTIMIZATION AND THE PROCESS CHALLENGE DEVICES

6.1 Process optimization

Cycle design studies play a crucial role in the

optimization of the sterilization process, particularly in minimizing the turnaround time required to get the product to market. Mathematical modeling of sterilization and aeration processes allows controlling each phase and, consequently, it is possible to attain the reduction of the overall process time. Additionally, one should consider the equipment used and the product being sterilized, since these factors dictate the process variables definition.

Traditionally, the process time of EOG sterilization is greatly influenced by two operations. These are microbiological analysis and aeration time. The implementation of parametric release eliminates sterility test (14 days cultivation after sterilization against sterilized MDs), but require tough requirement to determine several factors associated EOG sterilization. The validation of sterilization and aeration processes, with consequent assessment of EOG residues in compliance with the requirements of ISO 10993-7, ISO 11135-1, ISO 11135-2 and AAMI TIR 16.

The microbiological qualification approaches should also be considered as a part of process optimization, since the attained cycle is influenced by the methodology considered.

6.2 Process equivalence

Process equivalence is a method used to assess sterilization by different equipment, minimizing the number of tests required to qualify the process. The particular requirements that should be followed and the studies involved for assuming process equivalence, and consequently a reduced performance qualification (PQ), are described in AAMI TIR 28 (AAMI TIR 28, 2009).

6.3 Sterilization load and the process challenge device

The sterilization load with the highest density (and with the lowest thermal diffusivity), represents a sterilization challenge (AAMI TIR 16, 2009) and these products are usually elected for EOG processing. It is important to analyze the challenge (in terms of lethality) that the MDs under consideration present to the sterilization process.

Similar MDs can be grouped into product families. After product families are defined the most difficult-to-sterilize product in the family, which represents all devices in the group (family representative), should be identified. The master process challenge device (PCD) will be the worst-case product, or representative member, of the multiple-product families and it should be selected to challenge the sterilization process. The PCD packs may be a user-assembled test pack or a commercially available pre-assembled test pack (http://multimedia.3m.com/mws/media/381381O/biological-indicator-process-challenge-devices.pdf). Its selection can be done by a sterilization specialist evaluation (considering it estimated resistance to EOG sterilization) or after some testing, which of several products is more difficult-to-sterilize. This testing usually includes a thermodynamic (temperature and humidity response of the load) and a comparative microbial resistance study that shall comprise at least one fractional cycle run.

By placing the BI (e.g., paper strip) within the most difficult to sterilize location in the sterilization chamber is identical concept of PCD. PCD should place in the easiest place to retrieve in the chamber, not in the most-difficult-place to sterilize. All products within this EOG processing should present an equivalent challenge to the sterilization process when compared with the PCD. The product design and complexity, its composition, its microbial load, resistance of product and packaging to the sterilant gas diffusion, pallet density of the product (due to the temperature and absorption characteristics) and the desired SAL must be evaluated.

The same procedure should be followed when adopting a new or altered device (and/or packaging) into an existing validated sterilization process. The currently validated product or PCD would then be used as the basis for comparison with any candidate product. If the candidate product represents a greater challenge to the sterilization process than the PCD, a PQ should be reperformed in accordance with AAMI/ISO 11135-1: 2007. In that changing case, the user needs to be approved by the authority in Japan. The AAMI TIR 28 is a useful guide for minimizing the risk of introducing a new or modified product that represents a greater challenge to the sterilization cycle than the one previously validated. All changes need approval from authority in Japan because it is differed from the approved procedure.

6.3.1 External process challenge device

External process challenge devices (EPCD) are placed in the load but externally to the product, and are often used in routine processing to facilitate retrieval from the load after sterilization. An EPCDs resistance should be considered against the product bioburden that is being sterilized and the internal PCD (IPCD) because IPCD should resemble the most difficult-to-sterilize product within the internal loads in sterilization chamber.

The EPCD selection can be performed during cycle development and/or validation because it serves as a surrogate for the IPCD by demonstration during fractional exposures of a resistance greater than or equal to that of the IPCD (Rogers, 2005; ISO 11135-1, 2007; ANSI/AAMI ST 41, 2008; ISO 11135-2, 2008; AAMI TIR 28, 2009; AAMI TIR 16, 2009).

7. QUALIFICATION OF EOG STERILIZATION

Specific guidelines for validation of the sterilization processes, which includes physical and microbiological PQ, are developed and published by AAMI in conjunction with ISO. The validation of EOG sterilization processes is described in detail in ISO 11135 series (2007).

7.1 Protocol

A protocol, which outlines the overall validation requirements, must be prepared. The protocol should describe the MD and should specify the procedures to be followed during process validation and acceptance criteria (Rogers, 2005; ISO 14161, 2009).

7.2 Final report

A final report should compile all data, process conditions and test results that support process assessment.

7.3 Installation qualification and operational qualification

This topic will not be covered since the basis for its development is analogous to other similar processes.

7.4 Performance qualification

The performance qualification (PQ) consists of rigorous microbiological and physical testing to demonstrate the efficacy and reproducibility of the sterilization process. The microbiological performance qualification (MPQ) assures that the required lethality for the product/load combination in the sterilizer is achievable. The physical performance qualification (PPQ) is useful in defining reproducibility criteria while assuring product or package integrity. The PQ should be performed in the production chamber, setting one or more process variables (temperature, humidity and EOG concentration) at or below the minimum production routine levels, reducing the time in the preconditioning area, increasing the chamber loading time and the cycle starting time. This procedure assures safety of the sterilization cycle (ISO 11135-1,2007; AAMI TIR 16, 2009).

The ISO 11135-1 (2007) provides recommendations for preparing, placement and handling of PCDs or worst-case products, test samples and temperature and humidity sensors, and their minimum number depending on the vessel size. In addition, the minimum number of PCDs depends on the MPQ method chosen. The minimum number of cycle runs is also described in ISO 11135 or each specific method.

One should consider the representative product locations/sites through the load that challenge the sterilization process (i.e. the most difficult-to-sterilize locations) to ensure that a required SAL is attained (ISO 11135-1, 2007; AAMI TIR 16, 2009).

7.5 Routine monitoring and control

After validation study of the sterilization process, adequate procedures to be routinely followed must be defined. Specifications must describe the sterilization process aspects necessary to assure conformance with the validated cycle and to be maintained with an established change control procedure. The conformity with the specified process parameters must be attained; otherwise, product cannot be released as sterile (ISO 11135-1, 2007).

The conventional traditional release method requires that the process parameters are within the validated tolerance and that the BIs exposed to the sterilization process are inactivated. The parameters are the recorded raw data and evaluated process parameters obtained at the validation study. The equipment potentialities are enough to evaluate the impact of process parameters on microbiological inactivation (AAMI TIR 20, 2001). The physical monitoring provides real-time assessment of the sterilization cycle parameters and it is essential to detect the eventual malfunctions early, so that appropriate corrective actions can be taken (ANSI/AAMI ST 41, 2008).

7.6 Parametric release

Parametric release can only skip sterility test before shipping and in the real status humidity determination is quite difficult due to EOG polymerization, which result in poor reproducibility of humidity determination.

Parametric release is the assessment of sterilization adequacy based on physical parameters measurement. If a sterilization cycle operating within specified tolerances has been shown to be both effective and reproducible, confirmation that the process parameters were within tolerance is taken as evidence of cycle reliability. The requirements for validation and routine control are more stringent. These requirements are outlined in ISO 11135 (2007) and guidance can be found in AAMI TIR 20 (2001). The direct analysis of humidity during conditioning and EOG concentration during sterilant exposure time are key parameters and sufficient equipment to correctly and reproducibly determine humidity in EOG environment is not available and EOG polymerized in humidity sensor (AAMI TIR 20, 2001; ISO 11135-1, 2007). This procedure enhances operational efficiency and is also of economical interest for the healthcare market, since it decreases the running costs. However, it is risky to ship only physical parameters and determination of humidity is problematic. In addition, aeration is additionally required before shipping even tough 14 days sterility test may eliminate. In Japan, most of MD

manufacturers do not use parametric release, because authority approval is additionally required and during aeration, sterility test can be conducted. It cannot ship immediately after EOG sterilization due to aeration, so parametric release is more appropriate for autoclave sterilization rather than EOG sterilization. EOG sterilization has too many factors to be estimated and humidity factor is most-difficult to determine reproducibly, thus as a whole parametric release is risky. For gamma-ray irradiation, it can be carried out dosimetric release. This is because gamma-ray irradiation is required only doses as a major parameter, so dosimetric release in place of parametric release can be approved and conducted in ISO 11137 series (2012).

7.7 Maintaining process effectiveness and re-qualification

A periodic re-qualification study is recommended at least every two years and, preferentially, every year. If no substantial changes occurred in the process or materials, a documented evaluation review may be sufficient to verify that nothing that would affect the process has changed. Some specialists recommend a confirming cycle to increase the reliability of this evaluation. This review should also demonstrate that the resistance of the product bioburden has not increased to a level that would invalidate the use of the PCD or compromise the SAL claim of the process. If the process changes, the authority approval is required in validation study.

For parametric release, revalidation must be performed annually. Additionally, re-qualification should be conducted after relocation, any major redesign of the sterilizer, sterilizer malfunctions and major repairs (ISO 11135, 2007; ANSI/AAMI ST 41, 2008; AAMI TIR 16, 2009). These changes are also required authority approval.

8. CONTRACT STERILIZATION

Contract sterilization grows due to the increased requirements related to EOG sterilization. The responsibility for sterility is shared by the MD manufacturers and the contract sterilization facilities. Therefore, it is essential that the division of responsibilities is clearly defined and understood by both parties to ensure a well-controlled sterilization process.

AAMI TIR 14 provides additional guidance on this topic and, in particular, gives guidelines for manufacturers' selection of a sterilization facility and for the written agreement that must be established between product manufacturer and contract sterilizer. Written agreement should define the responsibilities of each part related to the sterilization process and should establish the handling procedures to be adopted (AAMI TIR 14, 2009).

9. CONCLUSION

Sterilization by EOG results after diffusion of effective density of humidity and gas into the innermost sections of MD. This diffusion process is impacted by temperature, the number of barriers to the penetration of the gases (layers of packaging), by the case density of the product, and by the complexity and design of the device itself. Therefore, a person knowledgeable in the chemistry of EOG sterilization and its effect on the lethality of microorganisms should perform the following reviews; an understanding of how the process works is necessary to identify the product that will eventually be used to challenge the sterilization process.

For efficient and cost-effective validation performance, prior product and process evaluation is suggested. If the facilities produce a wide range of sterile products, similar devices can be grouped into families. A family of products can be considered to be all those products of similar design and materials of construction, but consisting of different sizes, i.e., all Foley catheters, sized 8 French to 16 French. After family groups are determined, select the most difficult-to-sterilize representative product in the family to represent all the devices in the group. If the evaluation results in multiple product families, it is advisable to select from the representative products, a single most-difficult-to-sterilize product that will be used as the master PCD. The BI will be placed within the most difficult-to-sterilize interior spaces of the master challenge device and be used to ensure that the sterilization process delivers the desired sterility assurance level (SAL) of 10^{-6}. By this sterility assurance can be confirmed in success.

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